Histiocytoses of childhood
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Histiocytoses

Histiocytoses are characterized by infiltration of various tissues and/or systemic manifestation by members of the mononuclear phagocyte system (MPS) or the dendritic cell system, outside the context of inflammation and metabolic storage disease.\[1-3\] A general introduction to the histiocytoses is given in Chapter 1. The designation ‘histiocytoses’ is based on historical grounds and suggest that these disorders are derived exclusively from fixed tissue-macrophages. However, histiocytoses comprise diseases, as defined above, of cells of both the monocyte/macrophage cell system and the dendritic cell system. These cell lineages have different but overlapping functions: the main function of macrophages is phagocytosis, the main function of dendritic cells is antigen-presentation (or ‘immunoregulation’). It has been suggested that MPS would be renamed M-PIRE system (mononuclear phagocyte and immunoregulatory effector) joining the two cell systems and appreciating both distinct functions.\[7\] The histiocytoses are traditionally divided into 3 classes. Class I histiocytoses includes only Langerhans’ cell histiocytosis. Class II histiocytoses are considered ‘reactive’ histiocytosis of cells with non-Langerhans’ cell features, and class III comprise the malignant proliferations of monocytes, macrophages, as well as dendritic cells. Based on the two ‘archetype’ functions of members of the M-PIRE, it is reasonable to assume that the etiology of histiocytoses may reflect these distinct functions. Histiocytoses originating from the dendritic cell system are more likely to develop be related to defects or insufficiencies in antigen-presentation or subsequent effector mechanisms, whereas histiocytoses of macrophages are more likely to be related to insufficiencies of phagocytosis. Classification of histiocytoses may reflect these two cell systems and possibly the two etiologic mechanisms. Histiocytoses could then be maybe better if classified in a different way. Class I is then suggested to comprise histiocytoses originating from the dendritic cell system, class II could include histiocytoses of predominantly phagocytic mononuclear cells, and class III the malignancies of both cell lines.

Juvenile xanthogranuloma

One of the current class II histiocytoses is juvenile xanthogranuloma (JXG), which is thought to originate from macrophages. In Chapter 2 of this thesis an immunohistochemical study to the nature of the cells involved in JXG is described. Remarkably, the cells in JXG show expression of CD1a. This cell membrane molecule is strongly associated with the dendritic cell lineage and is expressed by Langerhans’ cells and their precursors, the so-called ‘indeterminate’ cells that express S-100 (present on Langerhans’ cells) but they lack Birbeck granules. \[67,194,195\] S-100 and Birbeck granules are not found in JXG cells. The expression of CD1a suggests that JXG cells are related to indeterminate cells and subsequently to Langerhans’ cells. In contrast, macrophage related markers including CD68 are expressed by JXG cells. Within the dermis, the site of most JXG lesions, there is a population of ‘macrophages’ with constituent expression of MHC class II,
called dermal dendrocytes.[131-133] These cells have potent antigen-presenting capacities. It has been suggested that the dermal dendrocytes interact with perivascular T-lymphocytes to generate immune responses when antigens are delivered to the dermal microvascular unit from the circulation.[130] After isolation, some dermal dendrocytes express CD1a, and this population may include Langerhans' cell precursors.[136,137] Speculatively, dermal dendrocytes are activated by a local stimulus, resulting in a lesion with accumulation and/or proliferation of cells histopathologically identified as JXG therewith explaining both dendritic cell markers and macrophage markers. The nature of this stimulus remains unknown, but may be an antigenic stimulus, most likely originating in the dermis, since JXG lesions are usually solitary. In some cases, JXG lesions are located outside the dermis in deeper localizations. It is possible that CD1a positive dermal dendrocytes are capable of migration, and result in some cases of deep-seated JXG. In keeping with the dermal dendrocyte origin of JXG, a recent study showed JXGs to be consistently positive for factor XIIIa (B.E. Favara, personal communication).

Many phagocytic cells including 'foam cells' are present in JXG lesions. These cells may seem contradictory with the proposed 'antigen-presentation etiology' of JXG. However, antigen-presenting cells are also capable of phagocytosis and endocytosis, although they are not as efficient as are phagocytes. Since the cells in JXG are related to macrophages, some phagocytic activity explaining the presence of foam cells may still be present.

Since JXG may be derived from cells of the dendritic cell system, it should be included in the class I histiocytoses, along with Langerhans' cell histiocytosis.

An important differential diagnosis of JXG is Langerhans' cell histiocytosis. According to the Histiocyte Society, immunohistochemical detection of CD1a expression on the cells within the lesion is required to make a definitive diagnosis of Langerhans' cell histiocytosis. The expression of CD1a by JXG cells, however, indicates that the expression of CD1a should be carefully used in establishing the histopathological diagnosis. To better distinguish JXG from Langerhans' cell histiocytosis, the immunohistochemical evaluation of a panel of macrophage and dendritic cell markers, among which CD68, factor XIIIa, and CD1a, should be considered when fresh frozen material is available. S-100 is a valuable marker on paraffin-embedded tissue.

Langerhans' cell histiocytosis and normal Langerhans' cells

Expression of cellular adhesion molecules on Langerhans' cell histiocytosis cells

Langerhans' cell histiocytosis (LCH) is characterized by an accumulation and/or proliferation of Langerhans' cells (LCs).[5,8,138] Normal LCs are present in epidermis, mucosa, lymph node and thymus. LCH lesions, however, occur at different sites. Aberrant migration and homing of Langerhans' cells may therefore play a role in the pathogenesis of LCH. In Chapter 3 and Chapter 4, the expression of cellular adhesion molecules on LCH cells and other cells within the lesions is investigated. This expression was compared with LCs in epidermis and lymph nodes.
showing a dermatopathic reaction, thought to reflect normal resting LCs and activated, migrated LCs, respectively.

LCH cells express CD1a, S-100 and possess Birbeck granules resembling epidermal LCs. Activation of LCs is associated with loss of Birbeck granules and CD1a expression. We found the LCH cells, like epidermal LCs, to express CD62L that is not expressed by activated LCs. The integrins α5 and α6 were not expressed by epidermal LCs but were highly expressed by activated LCs and could not be demonstrated on LCH cells. In contrast, LCH cells strongly expressed adhesion molecules CD54, CD58, CD49d, and the β1- and β2-chains of integrins that are only expressed during activation of normal LCs.[72,90,95,96] Remarkably, some adhesion molecules that are not found on normal LCs at all, CD2, CD11a and CD11b, were demonstrated on LCH cells.

These data suggest that expression of cellular adhesion molecules on LCH cells is abnormal when compared to normal LCs. Although site-specific pattern of adhesion molecule expression could not be demonstrated on LCH cells or endothelial cells in the lesions, aberrant expression of adhesion molecules on LCH cells may contribute to abnormal migration of LCs in LCH, resulting in lesions located at sites other than normal sites of occurrence of LCs. The abnormal expression of cellular adhesion molecules could facilitate firm homotypic interactions between LCH cells in lesions.

Some conclusions can be drawn from Chapters 3 and 4 regarding the relation between LCs and LCH cells. LCH cells express pertinent characteristics shared with normal epidermal LCs. Our data, however, suggest that LCH cells also express markers of activated LCs, cells capable of migration. Finally, LCH cells express adhesion molecules that are not expressed by either epidermal or activated LCs. The cellular adhesion molecule phenotype of LCH cells has characteristics of both resting, epidermal LCs and activated LCs. This may be indicative of an arrested state of activation and/or differentiation of LCH cells, possibly also reflected by the expression of adhesion molecules not expressed by normal LCs. This arrested state of activation and/or differentiation could be related to an immunologic dysregulation, that has been suggested to underlie LCH.[5]

Presence of cytokines in Langerhans' cell histiocytosis lesions

A central feature of normal immunologic regulation involves the production and local action of cytokines. In Chapter 5 and Chapter 6 we investigated the presence of cytokines, known to be produced by or to influence normal LCs, in LCH lesions using immunohistochemistry.

In Chapter 5 cytokines are investigated in a specimen from a patient with pulmonary LCH who had unilateral lung transplantation for extensive pulmonary interstitial fibrosis. The pulmonary LCH lesions contained IL-1α, IL-4, bFGF and GM-CSF. GM-CSF was not found in the LCH cells but most likely in macrophages, and in interstitial matrix.

In Chapter 6 we report a more extensive panel of antibodies used to investigate the presence of cytokines in a variety of LCH lesions from different localizations. For comparison with normal LCs, sections of normal skin and lymph nodes with dermatopathic reaction were also studied. We found LCH cells to stain for IL-1α,
SUMMARY & GENERAL DISCUSSION

IL-1β, GM-CSF, TGF-α, TGF-β, TNF-α and IFN-γ. Epidermal LCs were found to stain for IL-1α, IL-1β, GM-CSF, TGF-α, TGF-β, TNF-α, IFN-γ and bFGF. In contrast, normal activated LCs in dermatopathic reaction were negative for these cytokines. Some of these cytokines are of special interest in LCH lesions. The presence of IL-1α, IL-1β, and TNF-α, may activate osteoclastic bone resorption resulting in osteolysis, a frequently observed phenomenon in LCH lesions of bone. TGF-β has been strongly related to fibrosis. Fibrosis may be a prominent finding in end-stage LCH lesions and the development of diabetes insipidus in LCH of the central nervous system (CNS) has also been related to fibrosis. TGF-β may also play a role in LCH. GM-CSF has been identified in various tissues as a cytokine important in recruitment of LCs, and the presence of GM-CSF within LCH lesions may be important in the distribution of LCH cells. The combination of GM-CSF and TNF-α has been reported to be essential for development and viability of LCs. Speculatively, the abundant presence of these cytokines in LCH lesions may facilitate prolonged viability of LCH cells. We found TGF-α in epidermal LCs and also in LCH cells in contrast to its absence in activated LCs. TGF-α has many functions, including the promotion of epithelial proliferation. Speculatively, the abundant presence of TGF-α in LCH lesions may be an inducer of Langerhans’ cell proliferation.

Summarizing Chapters 5 and 6 regarding the relationship between LCH cells and normal LCs indicates that the pattern of cytokines in LCH cells resembles that observed in normal epidermal LCs. It seems likely that these cytokines are involved in the initial activation (and proliferation?) of normal LCs. In the normal situation this production is probably down-regulated once LCs start to migrate. The abundant presence of these cytokines in LCH cells may indicate that a ‘down-regulatory’ signal is lacking in LCH, resulting in an accumulation and/or proliferation of LCs. These cytokines also facilitate the variable and abnormal expression of cellular adhesion molecules on LCH cells we described in Chapters 3 and 4.

GM-CSF was present in LCH cells in most lesions. Although only one case of pulmonary LCH was available, the absence of GM-CSF in LCH cells in this case is intriguing. This could be due to the fact that we investigated an end-stage lesion, but other factors may also be involved. Solitary pulmonary LCH lesions usually occur in adult patients, and have been related to smoking. Pulmonary LCH is also characterized by distinctive intraluminal fibrosis and elastic fiber degradation. The absence of GM-CSF may indicate that pulmonary LCH lesions, and the cells involved, are etiologically different from LCH lesions elsewhere, although this needs further evaluation.

Proliferation, apoptosis and the expression of bcl-2 and P-gp in Langerhans’ cell histiocytosis

Proliferation of LCH cells has been demonstrated immunohistochemically. In addition, recent evidence indicates that LCH cells are of clonal origin. Clonality is necessary to define neoplasia, but not sufficient. We demonstrate in Chapter 7 the presence of proliferating LCH cells in LCH lesions. We also found that normal, activated LCs in dermatopathic lymph nodes are also proliferating. In
Chapter 3 and 4 we show that LCH cells in part resemble activated LCs, and therefore, proliferation of LCH cells should not be considered as abnormal per se. Speculatively, the suggested immunologic dysregulation in LCH may allow LCs to escape a 'down-regulatory' signal, resulting in a prolonged capacity to proliferate.

Little is known about loss of LCH cells in the lesions. We demonstrate the presence of apoptotic cells in LCH lesions and we found that LCH cells express bcl-2. Multinucleated giant cells also express bcl-2 at a stronger intensity than LCH cells. This may indicate that LCH cells and multinucleated cells, experience diminished susceptibility to apoptotic cell death, possibly contributing to the pathogenesis of LCH and in part explaining the chronic course of some lesions. Multinucleated giant cells could with their decreased susceptibility to apoptosis and by virtue of their cytokine production be a long-lasting influence on the microenvironment of the lesions, allowing LCH cells and other cells, such as macrophages and eosinophils, to accumulate and proliferate. This may also contribute to a chronic course of these lesions, in which macrophages contribute considerably to the number of cells.

P-gp functions as an energy dependent cell membrane efflux transporter, preventing high intracellular concentrations of certain chemotherapeutic agents. P-gp expression in LCH cells is reported in Chapter 7 and this expression may prevent accumulation of chemotherapeutics within the cell, thus providing a basis of drug-resistance. Interestingly, P-gp expression on LCH cells seems to be due to up-regulation of physiologically present P-gp, since we found P-gp in primary and recurrent lesions, and in normal LCs in epidermis. The latter may represent a mechanism to keep the adaptive immune system of the skin intact in a microenvironment where contact with toxins may be abundant. The successful use of etoposide (VP-16) in LCH, a drug known to be sensitive to P-gp-mediated drug-resistance, suggests that in most cases the clinical relevance of expression of P-gp in LCH (at least for this drug) is limited.

The increased expression in P-gp on LCH cells seems related to an event early in activation of LCs since normal LCs express P-gp, but LCs in uninvolved epidermis overlying lesions express P-gp with in higher numbers and with higher intensity. Activated LCs in dermatopathic reaction, however, lack expression of P-gp.

An hypothesis on the etiology and pathogenesis of Langerhans' cell histiocytosis; emphasis on the relation between Langerhans' cells and LCH cells

From the data presented in this thesis a possible relationship between the LCH cells and normal Langerhans' cells can be seen. LCH cells share defining characteristics with epidermal Langerhans' cells; expression of CD1a, S-100 and the ultrastructural presence of Birbeck granules. Normal LCs, when activated, migrate from the epidermis to the draining lymph node. Subsequent to activation and during migration, LCs experience several phenotypic changes, facilitating migration to the lymph node and presentation of antigen to T-cells; a decrease in CD1a expression, the loss of Birbeck granules and an altered expression of various adhesion molecules. Regarding the expression of cellular adhesion molecules, the LCH cells, indeed, resemble resting, epidermal Langerhans' cell to some degree. In contrast, the LCH cells also express adhesion molecules, at an intensity, like found in activated LCs. LCH cells also express adhesion molecules that are not found on normal
SUMMARY & GENERAL DISCUSSION

LCs at all. Together these data suggest that LCH cells suffer impeded activation, resulting in a phenotype that is intermediate between the resting, epidermal Langerhans' cell and the activated LC in dermatopathic reaction. This phenotype of aberrant adhesion molecule expression allows LC migration to lymph nodes, and possibly other sites. LCH cells produce the cytokines that are also present in normal epidermal LCs but normal activated LCs in dermatopathic reaction do not express these cytokines at an immunohistologically undetectable level. The hampered or partial activation of LCH cells results in continuation of an 'epidermal' cytokine profile suggestive that a 'down-regulatory' signal is lacking in LCH cells. This results in continuous production of these cytokines. Some of the cytokines we found to be abundantly present in the lesions are essential for viability and activation of LCs, therewith in part explaining the phenotype of LCH cells. Both epidermal LCs and activated LCs in dermatopathic reaction are proliferating. Since normal LCs in the process of activation and migration conserve a proliferating capacity, the proliferation of LCH cells is of an indication for genetic alterations per se.

Speculatively, LCH results from immunoregulatory insufficiency affecting Langerhans' cells. Activation of epidermal LCs and subsequent migration to the draining lymph node may be subject to improper 'handling' of a currently unknown antigenic stimulus as a result of this dysfunctional immunoregulation in LCH. Recent attention has focused on the involvement of superantigens as preliminary data indicate the presence of Vβ-restricted T-cells in the lesion (G. Kannourakis, 6th Niklas Symposium, 1995) The improper 'handling' results in aberrant and inconsistent expression of cellular adhesion molecules causing abnormal homing and arrested differentiation to a fully antigen-presenting phenotype with proliferation and a lack of allogeneic antigen-presenting function. These cells may escape additional 'down-regulatory' immunologic signals, through expression of bel-2. The production of cytokines by these LC-like cells may be altered or uncontrolled, resulting in prolonged viability and activation of LCs, and activation of other immune cells. In LCH lesions, the inconsistent expression of adhesion molecules and the abundant presence of cytokines may facilitate aberrant strong homotypic adhesion and contribute to extended LCH cell viability and the development of auto- or paracrine loops. Cytokine production may also cause some the induction of multinucleated giant cells to become long-lasting and continuous promoters of the LCH process. The nature and severity of the underlying immunologic dysregulation, and the nature of the hampered activation of LCs, may determine which organ systems are affected and to what extent, and how the disease is eventually controlled.

Future Prospects

In this study we used immunohistochemistry to demonstrate the expression and presence of a variety of molecules, including cytokines. The cytokines in LCH lesions should be further evaluated by detecting not only proteins but also by detecting mRNA coding for cytokines. Of special interest is the study to the expression of cytokine-receptors by LCH cells, since these receptors determine to which cytokines the cells respond.
To further and more accurately compare LCH cells and normal LCs, investigation of the normal biology of LCs is of utmost importance. Is (clonal) proliferation of LCs a physiologic event in the activation of these cells? Is there (oligo)clonality in normal, activated LCs? Do LCs express bcl-2 in a stage of normal development? Since most data concerning expression of adhesion molecules in LCs are derived from in vitro experiments, what adhesion molecules are (temporarily) expressed or up-regulated during activation and migration in vivo? What additional cytokines are produced in vivo by normal LCs, both resting and activated? LCs in various pathologic processes should also be investigated.

Furthermore, CD1a expression is generally associated with antigen-presenting cells but the exact function of CD1a is still obscure. Identification of this function may reveal whether this assumption of CD1a being an antigen-presenting cell system-specific marker is correct.

Obviously, the establishment of a LCH cell line (and a LC cell line) provides great opportunities for investigating the biology of these cells including the effects of cytokines and the mechanisms of possible multi-drug resistance. It is likely that specific drug resistance may also play a role in LCH.

The development of an animal model of LCH would provide opportunities to study the biology of LCH cells, especially with regard to homing and migration of these cells.

The expression of bcl-2 in multinucleated giant cells is of interest. Is this a property of multinucleated giant cells in LCH or is it a general prerequisite for multinucleated giant cell development? Investigating multinucleated giant cells in other diseases may be revealing.

The hypothesis of the pathogenesis of LCH is based on recent data concerning LCH cells and normal LCs that are described in this thesis. However, it proposes that some events are occurring in LCH that are not yet investigated. LCH according to this hypothesis may move through a poly- or oligoclonal stage to a monoclonal stage. Investigating clonality in early lesions, or in reactive lymph nodes, of patients with LCH may provide more information. A preliminary study suggests that epidermal LCs in large areas of epidermis may be clonal (A.C. Chu, personal communication). Effort should also be directed to the identification of genetic alterations in LCH cells, since the possibility of LCH being a neoplastic process is not ruled out. Investigating clonality in different and consecutive lesions from the same patient should be performed.

Only when the pathogenesis of LCH and the true nature of the LCH cells is known can an effective therapy be developed.